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LETHALITY OF PENTABORANE-9 IN MAMMALIAN ANIMALS

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FOREWORD

This study was initiated by Project TORES (Toxicology Research) under the sponsorship of the Advanced Research Projects Agency, DOD, and procured by the U. S. Army Chemical Center, Maryland. Upon termination of Project TORES, procurement and administrative functions were transferred to the Aerospace Medical Division, AFSC, Brooks AFB, Texas. Technical monitorship was delegated to the Toxic Hazards Branch, 6570th Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio. The research was performed in support of Project 6302, "Toxic Hazards of Propellants and Materials," Task 630202, "Pharmacology and Biochemistry," from 1 June 1962 to 31 May 1963. Dr. C. H. Wang was the principal investigator for Oregon State University and Drs. A. A. Thomas and K. C. Back were contract monitors for the 6570 AMRL.

The assistance of Dr. Lyle Calvin of the Department of Statistics, Oregon State University, in providing an analysis of the data on inhalation toxicity is greatly appreciated. Dr. T. D. Parsons of the Department of Chemistry has provided invaluable assistance with basic aspects of boron chemistry and high vacuum techniques. Our appreciation is also due Mr. Gilbert Butler for determinations of pentaborane-9 boron in these experiments.

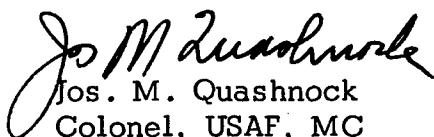
The experiments reported herein were conducted according to the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

ABSTRACT

A method of transfer and administration of pentaborane-9 to small animals was described. By use of this procedure, median lethal doses of pentaborane-9 by intraperitoneal and inhalation administration to the rat were determined to be 3.7 mg/kg and 0.42 mg/kg respectively. An intravenous median lethal dose to the rabbit of 0.52 mg/kg was estimated. The suggestion was made that pentaborane-9 does not oxidize appreciably prior to inhalation, and was almost quantitatively removed from respired air.

PUBLICATION REVIEW

This technical documentary report is approved.



Jos. M. Quashnock
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LETHALITY OF PENTABORANE-9 IN MAMMALIAN ANIMALS

I. INTRODUCTION

Several studies have been made on the physiological effects of pentaborane-9 upon mammals intoxicated by inhalation exposure. Typical symptoms of intoxicated human patients include central nervous system manifestations ranging from drowsiness to convulsions and alteration of electroencephalograph patterns (ref. 6 & 9). Similar effects have been observed with intoxicated dogs including changes in conditioned responses (ref. 15). Exposed humans (ref. 6) and experimental dogs (ref. 12) have produced elevation in blood sugar concentration and in nonprotein nitrogen. The latter observation may reflect some destruction of tissues. It has also been reported that with dogs exposed to pentaborane-9 in air, at a 40 ppm concentration for a period of 1.5 hours, cardiac arrhythmias, coronary insufficiency and depression of blood pressure were observed (ref. 12).

Exposure of various rodents to pentaborane-9 at 1 ppm for two weeks resulted in predominantly ataxic symptoms with rhinitis and loss of weight (ref. 5). Blood composition, nitrogen balance, renal function and liver function were found to be not significantly altered. The latter finding differs from earlier studies in which a slight impairment in liver function was noted (ref. 4) and excessive deposition of fat in the liver was reported (ref. 11).

Previous studies on the lethality of pentaborane-9 have employed a dynamic exposure technique, in which a mixture of pentaborane-9 and nitrogen, at a defined temperature and flow rate, was mixed with a large quantity of air and introduced continuously into an exposure chamber (ref. 14). The technique was necessitated by the pyrophoric characteristic of this compound.

In order to understand better the toxic action of pentaborane-9, it was desirable to obtain first hand information on the fate of this compound in intact animals. In view of the possibility that pentaborane-9 may be oxidized to some extent in air prior to its entry into the animal body or altered chemically in the lung, it was essential to devise a means to administer this compound in the liquid state into test animals by way of routes other than air inhalation.

In the present study, techniques have been developed to transfer liquid pentaborane-9 under an inert atmosphere to test animals via the intraperitoneal and intravenous routes. The perfection of such a technique permits one to examine the lethality of this compound on a more quantitative basis. The results obtained have also been examined comparatively with those observed in air exposure experiments. The latter include the estimated median lethal dose (LD₅₀) calculated from findings in published dynamic air exposure experiments and that observed in a static air exposure experiment designed in this laboratory.

II. MATERIALS AND METHODS

Experimental Animals and Materials

The pentaborane-9 used in the present study was manufactured by the Callery Chemical Company, Callery, Pennsylvania.

The rats used in these studies were males of the Sprague-Dawley strain, 350-450 grams in weight and were obtained from the Northwest Rodent Company, Pullman, Washington. The rabbits used were cross breeds of local origin, 1.7 - 2.2 kg in weight. Maintenance practice was in accord with accepted standards.

In view of the recognized chemical behavior of pentaborane-9 including its pyrophoric properties and its instability toward water, it was essential to handle this compound in a dry and inert atmosphere at all times. Consequently high vacuum techniques (ref. 8) have been used in the present study for the storage and the transfer of this compound. The determinations of purity of this compound have been carried out by manometric techniques with the high vacuum system. The high vacuum apparatus used was purchased from the Delmar Scientific Laboratories, Maywood, Illinois.

Pentaborane-9 was vacuum transferred from the steel shipping cylinder into a storage bulb of the high vacuum system using liquid nitrogen as the storage bulb coolant. A small aliquot of the compound was then vacuum transferred into the dispensing apparatus described below.

Microsyringe Dispensing Technique for Liquid Pentaborane-9

The determination of the toxicity of intravenously or intraperitoneally administered pentaborane-9 in small animals requires injection of liquid pentaborane-9 in microliter quantities. To facilitate this technique, a dispenser (figure 1) was developed which is similar to a liquid pentaborane-9 extraction device reported by Jensen and Goshgarian (ref. 3). The use of the dispenser permits pentaborane-9 to be vacuum transferred from the high vacuum storage bulb into the dispenser and then brought to atmospheric pressure with nitrogen. This technique yields liquid pentaborane-9 with a nitrogen covering atmosphere in the dispenser. A latex rubber-mercury port (figure 1) then permits repeated withdrawals of liquid into a microsyringe without an appreciable pressure differential between the microsyringe and the dispenser. Thus, a dry, nitrogen flushed microsyringe can be filled with liquid pentaborane-9 without exposure to the atmosphere.

The general technique used is described below.

The dispenser was attached to a manifold port of the high vacuum system and evacuated. Approximately 0.25 - 0.5 ml of pentaborane-9 was then vacuum transferred to the dispenser reservoir. While the pentaborane was still in the frozen state in the dispenser reservoir, pre-purified nitrogen was admitted to the high vacuum manifold portion to a pressure of 480-500 mm of Hg. The dispenser was then removed from the manifold after closing of the

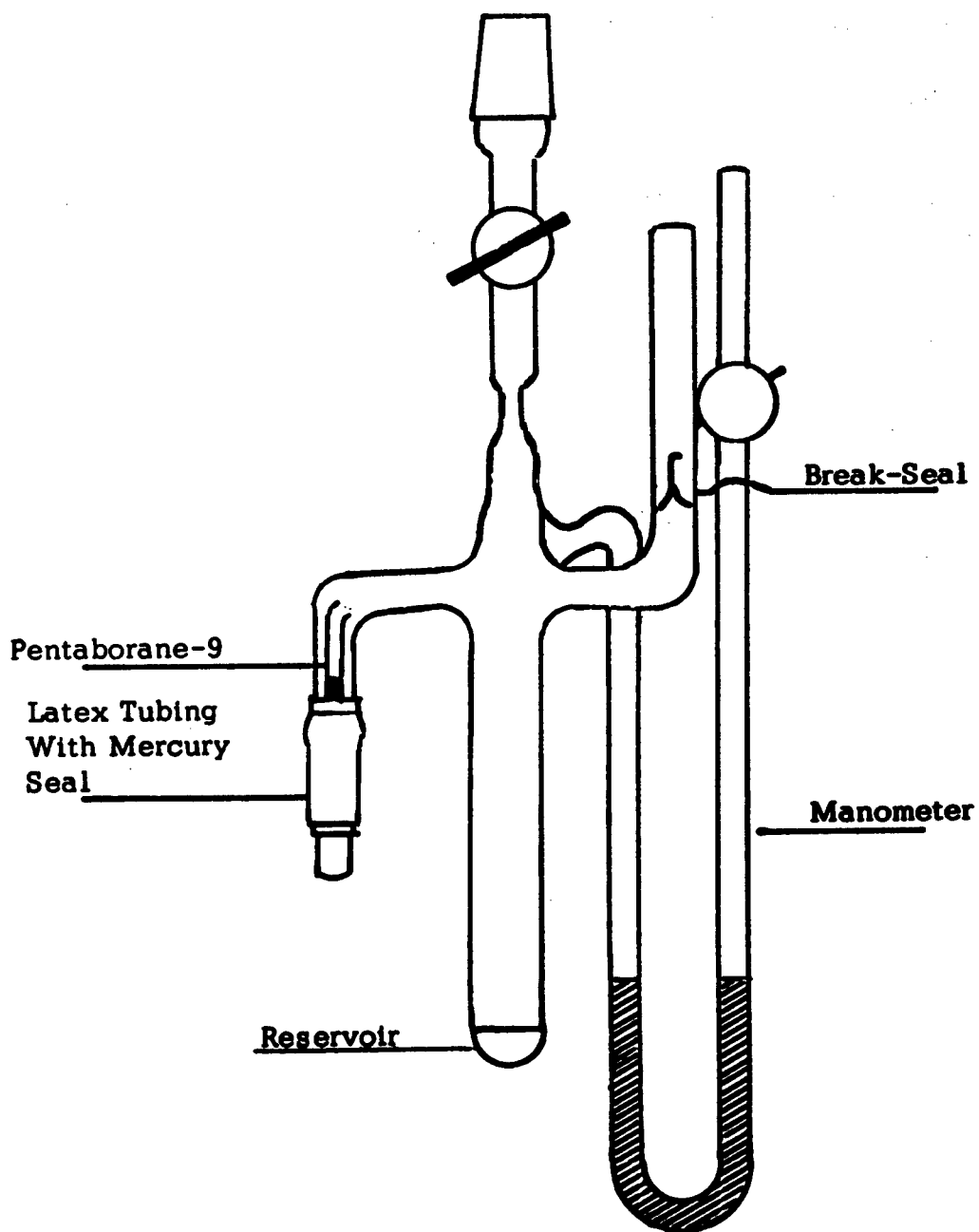


Figure 1. Dispensing Apparatus for Pentaborane-9

stopcock or flame closure. After the pentaborane reached room temperature the external arm of the Hg. manometer was flushed with nitrogen and carefully opened to equilibrate any pressure differential which might exist. The dispenser was then tipped slightly to decant pentaborane onto the mercury in the delivery sidearm.

A dry microsyringe was flushed with nitrogen and the needle inserted into the latex port of the delivery sidearm and into the pentaborane layer above the mercury seal. A desired amount of pentaborane-9 was drawn into the syringe. A slow stream of nitrogen was applied around the microsyringe needle as it was withdrawn from the latex port. The filled microsyringe was then used directly for intravenous or intraperitoneal injection of pentaborane-9 to the test animal. If it should be advantageous to decompose any residual pentaborane-9 in a dispenser, an alcohol-water mixture (1:1 v/v) can be placed in the break seal sidearm and the tip broken. Gentle hydrogen evolution will limit the rate at which the alcohol-water mixture is able to enter the dispenser.

Determination of the Median Lethal Dose of Pentaborane-9 Administered Via the Intraperitoneal Route.

The development of the transfer techniques described in the foregoing section made it possible to determine the lethality of pentaborane-9 administered to animals without prior contact to air. Such information is important since it is highly desirable to know whether the toxic action of pentaborane-9 is derived from the compound per se or derivatives such as oxidation products or hydrolysis intermediates.

In the experiments involving intraperitoneal administration, the determination of the median lethal dose (LD_{50}) was carried out with rats as test animals in duplicate experiments. In each of the two experiments, 16 rats were weighed and randomly distributed into four groups of four animals. The animals were caged alone and fasted for 12 hours prior to the administration of the fuel. The combined results were plotted on a probability scale as a dose response curve to indicate LD_{50} and standard deviation. The LD_{50} was also calculated by the method of Weil (ref. 13) and provided a similar value. Intraperitoneal microsyringe injections were made under an inert atmosphere.

Determination of the Median Lethal Dose of Pentaborane-9 Administered Via Intravenous Injection.

Since the intravenous injection of liquid pentaborane-9 into rats using the microsyringe technique is very difficult, rabbits were used as test animals in this regard. Defined doses, under an inert atmosphere, were injected into the marginal vein of the ear. The scope of this series of experiments was limited inasmuch as the primary purpose of the task was to detect whether a difference in lethality existed when pentaborane-9 was administered via intraperitoneal or intravenous routes.

Determination of the Median Lethal Dose of Pentaborane-9 Administered Via Inhalation.

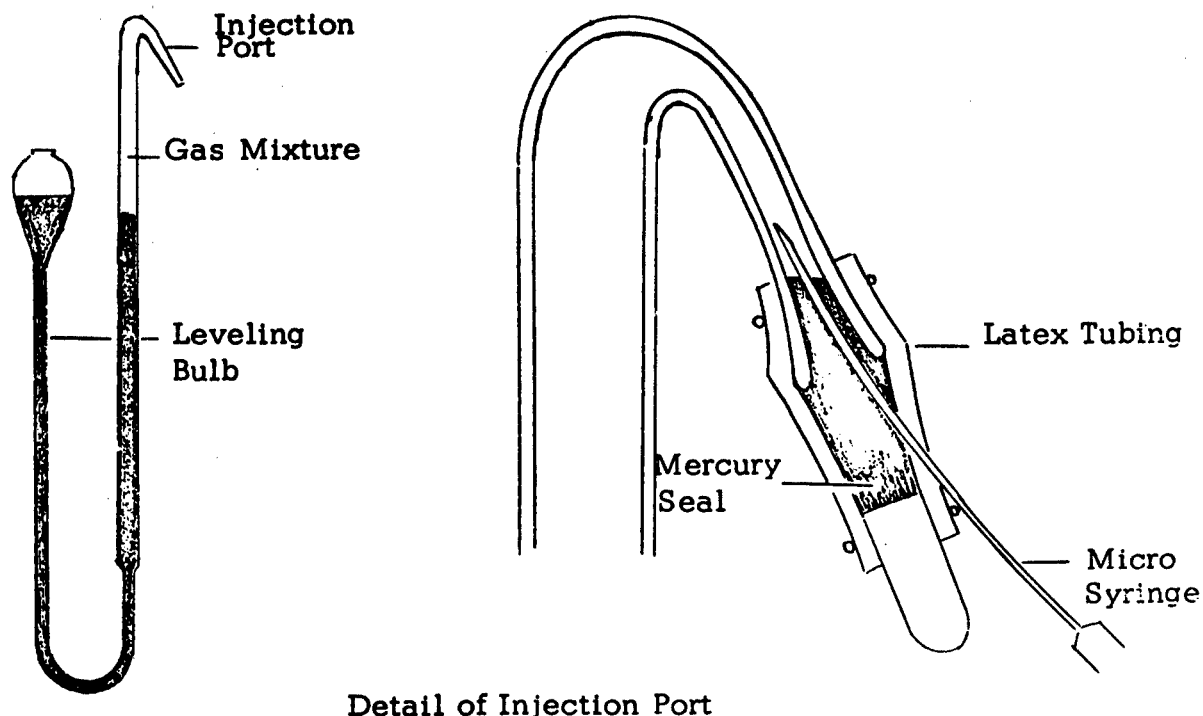
Previous work reported in this regard was concerned with inhalation experiments employing dynamic exposure techniques, in which a mixture of pentaborane-9 and nitrogen was introduced into a defined amount of air and passed continuously through an exposure chamber. Consequently, the net amount of pentaborane-9 absorbed by the test animal could not be ascertained directly. The administered dose was therefore calculated from the known pentaborane-9 concentration in air and the amount of chamber air involved in the respiratory activity of the animal. However, such a calculation must rely on the assumption that the pentaborane-9 in the inhaled air mixture has been completely absorbed by the lungs of the test animal.

In order to test the validity of the foregoing assumption, inhalation experiments in a static exposure chamber have been carried out to permit determination of the exact amount of pentaborane-9 absorbed from the enclosed atmosphere by the test animal. In view of the pyrophoric property of pentaborane-9, specially devised techniques have been developed to introduce safely a defined amount into a static exposure chamber.

The operation involves the use of a gas mixing chamber shown in (figure 2) where pentaborane-9 is mixed with nitrogen gas prior to being mixed with the air in the exposure chamber. The mixing chamber is essentially a vertically held gas buret 10 mm in diameter and 30 ml in total capacity. The upper end is tapered and formed into an inverted "U" tube, with a mercury sealed injection port at the free end.

Complete absence of oxygen in the mixing chamber is insured by admitting and expelling nitrogen gas through a hypodermic needle inserted in the injection port. After repeating this cycle several times the chamber is filled with nitrogen. The microsyringe technique described previously is used in introducing a given amount of liquid pentaborane-9 into the mixing chamber via the injection port. The preparation is allowed to stand for 24 hours to insure complete mixing by diffusion of pentaborane-9 vapor and nitrogen. Seven microliters of pentaborane-9 introduced into the mixing chamber presently in use gives a gas composition in the chamber of approximately 15 mg/100 ml of gas mixture. Chemical analysis of boron in each gas mixture is made according to the method of Hatcher and Wilcox (ref. 1).

In a typical exposure experiment, the test animal is placed in an 8 liter static exposure chamber, (figure 3). The chamber pressure is lowered to 50 mm of Hg. below the outside atmospheric pressure. A defined amount of gaseous pentaborane-nitrogen mixture in the mixing chamber is then transferred in a syringe (5 ml capacity and equipped with a 2-1/2" 22 gauge needle) from the mixing chamber into the static exposure chamber by way of its injection port. Since the chamber pressure at this time is still lower than the atmospheric pressure, it is possible to vent enough air into the chamber through the needle to insure quantitative transfer of the gas mixture. The chamber



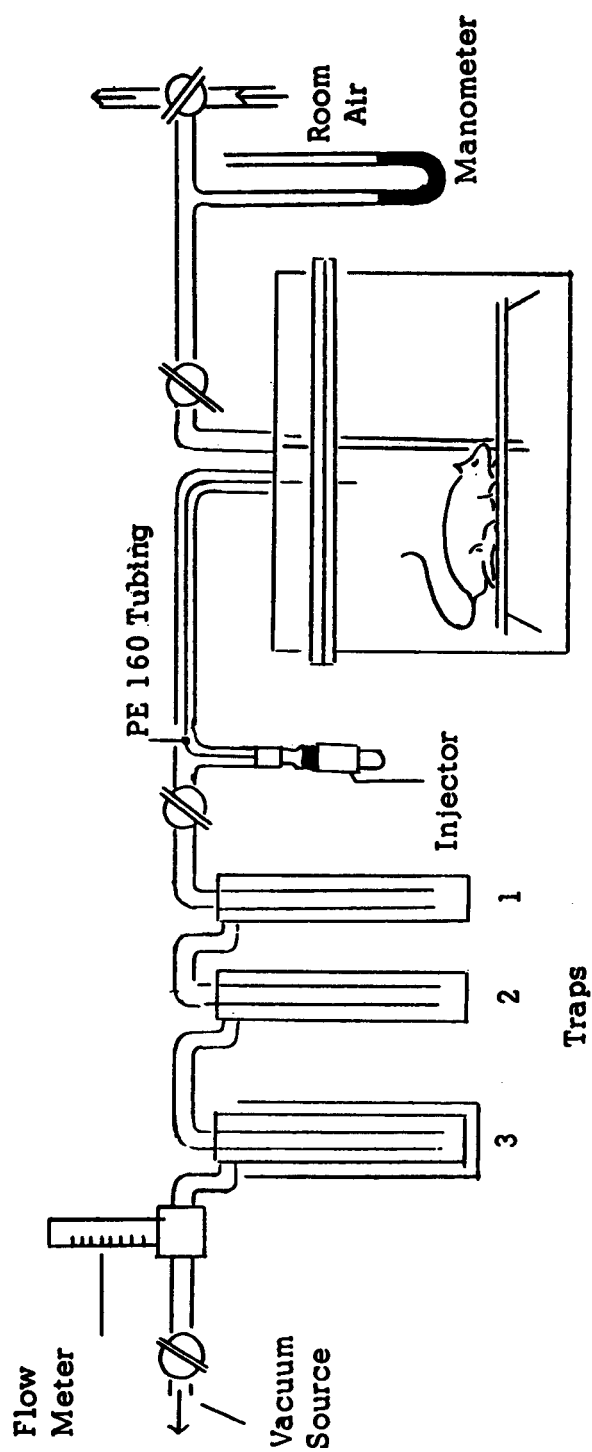
Detail of Injection Port

Figure 2. Closed Mixing Chamber for Pentaborane-9 Vapor and Nitrogen

pressure is then maintained at 10 mm Hg. below atmospheric pressure.

Forty minutes after the introduction of pentaborane-9 to the static chamber, the chamber atmosphere is swept out with several chamber volumes of air into two traps containing 2-ethoxy ethanol and a dry-ice acetone cooled trap in series. Residual pentaborane-9 in the exposure chamber is determined by chemical analysis of the boron content in the 2-ethoxy ethanol traps. The amount of pentaborane-9 retained by the test animal is obtained by the difference between administered and recovered boron and the results are expressed as mg of pentaborane-9/kg body weight.

In our experiments the amount of pentaborane-9 adsorbed on the chamber walls and tubing was determined to be negligible in control experiments without the test animal. Adsorption on hair of dead animals was found to be negative. Employing the static chamber technique it was possible to find the amount of pentaborane-9 absorbed by the test animals. The nature of the experiment does not permit design of a conventional lethality determination which calls for experiments employing a series of predetermined dosages.



1. 2-Ethoxy ethanol
2. 2-Ethoxy ethanol
3. Dry ice-acetone

Figure 3. Static System for Exposure of Small Animals to Pentaborane-9

Nevertheless, at the 68 percent confidence level a determination of the LD₅₀ of pentaborane-9 administered by inhalation was obtained by probit analysis of percentage of test subjects responding below each dose level.

III. RESULTS AND DISCUSSION

As shown in figure 4, the LD₅₀ of pentaborane-9 administered by intraperitoneal injection to rats was found to be 3.7 (2.8 - 4.6) mg/kg. This finding is contrasted sharply by the results of inhalation experiments shown in figure 5, i.e., LD₅₀ by inhalation = 0.42 (0.38 - 0.46) mg/kg. Dose and response information resulting from intravenous administration of pentaborane-9 indicates an approximate LD₅₀ by this route of 0.52 mg/kg (figure 6). This value was judged to be a reasonable approximation of the true LD₅₀ and considered of similar magnitude to the rat inhalation LD₅₀. The highest dose survived was 0.59 mg/kg and the lowest lethal dose was 0.46 mg/kg. Such a comparison is valid only when there is no species difference. The intravenous LD₅₀ of HEF-2, an alkylated derivative of pentaborane-9, is similar in rats and rabbits (ref. 2), and a similar relationship is assumed to hold for pentaborane-9.

A number of considerations arise from the differences in toxicity resulting from different routes of administration. In view of the apparently similar magnitudes of the intravenous LD₅₀ of pentaborane-9 in rabbits and the inhalation LD₅₀ in rats, it seems that a reaction such as air oxidation prior to inhalation is not a factor in pentaborane-9 toxicity. It may be expected that the rapid entry into circulation provided by inhalation or direct injection will facilitate transport of toxic material to sites of action, and may be responsible for much of the difference in lethality. In addition, any tissue reaction may localize the injected material at the site of deposition.

Also of interest, is the similarity of the LD₅₀ determined in the static chamber to the LD₅₀ calculated from observations made in inhalation experiments with a dynamic exposure chamber by other workers (ref. 14). On the basis of this similarity, it appears that at an air concentration of 10-20 ppm, the pentaborane-9 in the inhaled air is completely absorbed through the lung of the rat. This is not surprising in view of the lipid nature of the alveolar surfaces. The calculated LD₅₀ of 0.35 mg/kg is derived from the assumption that the pentaborane-9 of the respired chamber atmosphere is completely absorbed by the lung of the subject (refs. 10, 14).

The exact fate of the pentaborane-9 in test animals cannot be ascertained at this time. In view of the slow rate of hydrolysis* it is possible that in spite of the apparently short biological half time suggested

*Vapor Phase Hydrolysis of Pentaborane-9 by G. W. Butler, T. D. Parsons, D. J. Reed, and C. H. Wang.

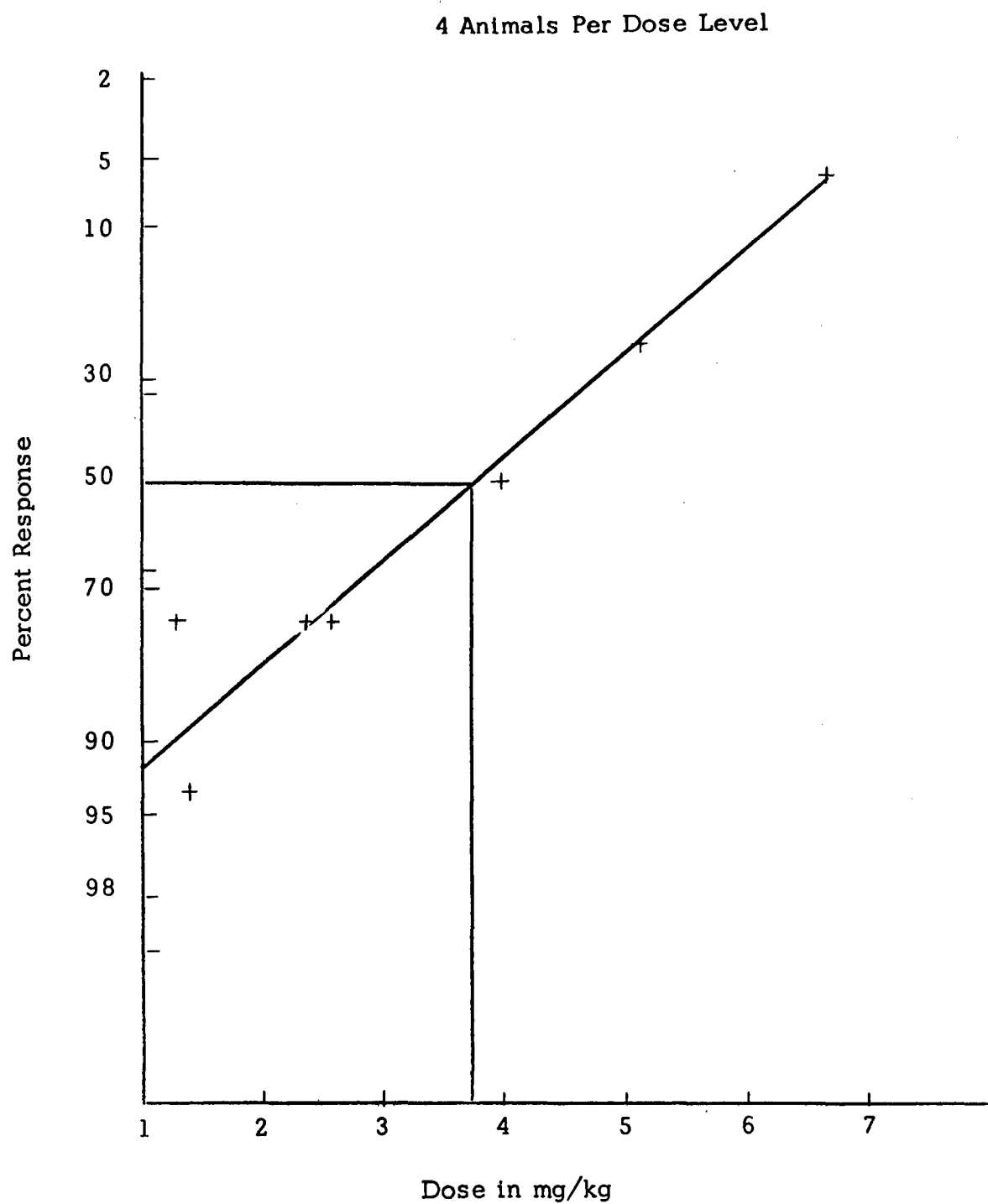


Figure 4. Dose-Response Effect of Pentaborane-9 Administered Intraperitoneally to the Rat

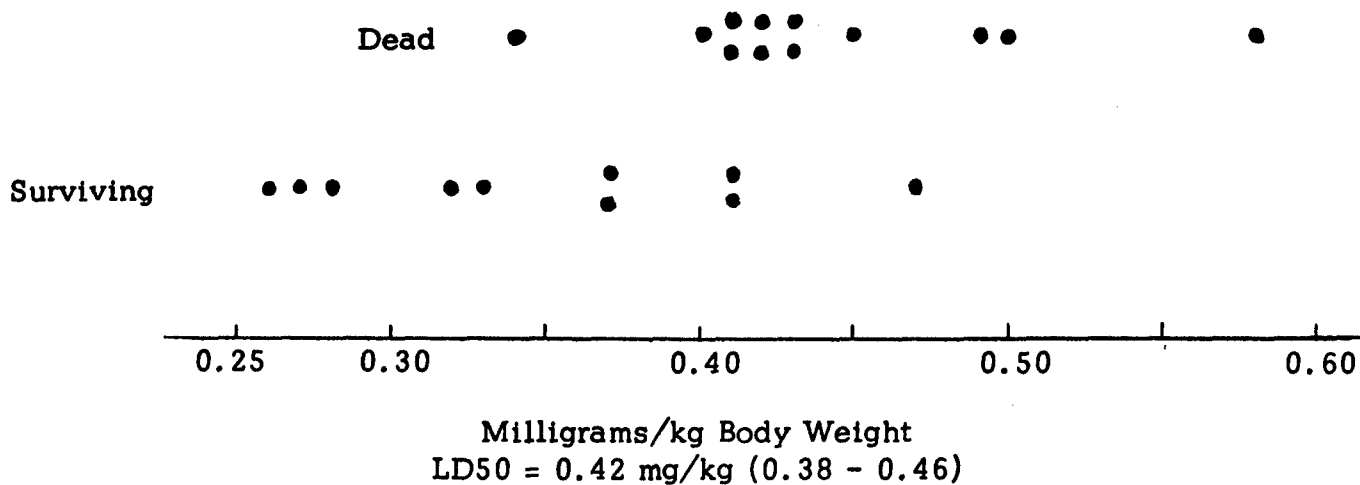


Figure 5. Lethality of Pentaborane-9 by Inhalation in the Rat

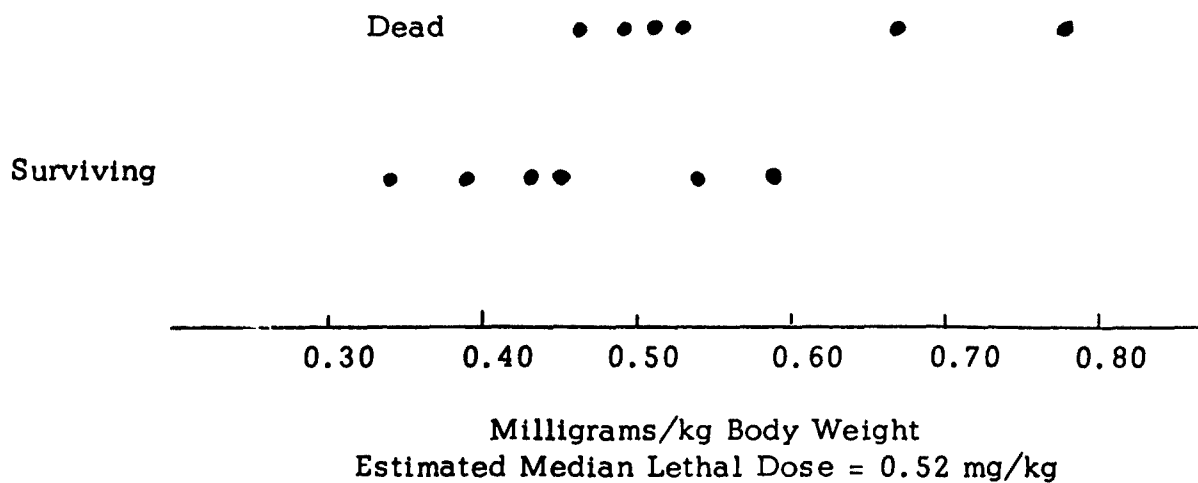


Figure 6. Lethality of Pentaborane-9 by Intravenous Administration to Rabbits

by the increase of effective concentration with time (ref. 14), that intact pentaborane may remain in the body, in either an aqueous phase or dissolved in fatty materials, for a considerable period. Such a retention may be characteristic of all boranes. Miller et al, have found that detectable borane remains in brain and liver tissue after 28 daily injections of HEF-3, at levels of 100 μg and 250 $\mu\text{g}/\text{kg}$. These animals were asymptomatic and no serum borane was detectable. Preliminary experiments carried out in this laboratory with pentaborane-9- H^3 have revealed that pentaborane-9 may act as a reducing agent in the body as evidenced by the retention of 5-10 percent of the administered tritium in the body fluid. This tritium is not ionizable nor removable by repeated distillation.

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